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Review

Vaccination of domestic ducks against H5N1 HPAI: A review

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ABSTRACT

Domestic ducks play an important role in the epidemiology of H5N1 highly pathogenic avian influenza (HPAI) viruses. Consequently, successful control of H5N1 HPAI in ducks is important for the eradication of the disease in poultry and in preventing infections in humans. Domestic ducks, however, include different species and breeds, and the susceptibility to infection, disease and response to vaccination can vary depending on the species and age of the bird. Most domestic duck species are descendants of mallard ducks (*Anas platyrhynchos*), but in Asian countries Muscovy ducks (*Cairina moschata*) are also commonly farmed. Current vaccines and vaccination practices are insufficient for the control of H5N1 HPAI virus infections in domestic waterfowl and new vaccination strategies are needed. Although vaccination has proven effective in protecting ducks against disease, shedding of the virus still occurs in clinically healthy vaccinated populations. To improve protection of ducks against H5N1 HPAI, vaccination programs must take into account the susceptibility of ducks to circulating viruses and the particular production systems and husbandry practices of the country. Vaccination needs to be implemented as part of a comprehensive control strategy that also includes biosecurity, surveillance, education and elimination of infected poultry.

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1. Background on control of H5N1 HPAI in domestic ducks

Waterfowl are one of the principal natural reservoirs of avian influenza (AI) viruses (Stallknecht, 2008; Swayne and Halvorson, 2008; Webster et al., 1992). Historically, ducks naturally or experimentally infected with most AI viruses, including highly pathogenic avian influenza (HPAI) viruses, only develop subclinical to mild

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disease (Alexander et al., 1986; Cooley et al., 1989; Perkins and Swayne, 2002; Shortridge et al., 1998). This premise has dramatically changed since 2002, as many Asian lineage H5N1 HPAI viruses have been shown to replicate systemically and produce mortality in ducks [reviewed in Pantin-Jackwood and Swayne, 2009] (Bingham et al., 2009; Guionie et al., 2010; Pfeiffer et al., 2009; Phuong do et al., 2011; Tang et al., 2009). The age and species of the ducks also affects the outcome of the infection, with younger ducks and some species, like Muscovy ducks, being more likely to show clinical signs (Brown et al., 2006; Cagle et al., 2011; Londt et al., 2010; Pantin-Jackwood et al., 2007, 2012; Phuong do et al., 2011). Host immune responses certainly play a role in the differences observed in pathogenicity; however, not much is known about the immune response of ducks to AI virus infection (Magor, 2011). The differences observed in pathogenicity of H5N1 HPAI viruses in domestic ducks has implications in surveillance and control of the disease, as asymptomatic or mildly symptomatic infected ducks are difficult to recognize and can spread the virus to other susceptible poultry and threaten human health. The inability to use clinical disease as an indication of infection status allows the unrestricted movement of viral carriers, interferes with disease control, and promotes skepticism among farmers about the disease threat.

Although asymptotically infected wild migratory ducks are suspected of contributing to the spread of H5N1 HPAI viruses from Asia to other parts of the world (Cattoli et al., 2009; Keawcharoen et al., 2008; Kim et al., 2009), domestic ducks are more likely perpetuating H5N1 HPAI viruses in nature (Hulse-Post et al., 2005; Kim et al., 2009; Sturm-Ramirez et al., 2005). Free-range, as well as backyard domestic ducks have been associated with disseminating H5N1 HPAI viruses (Gilbert et al., 2006; Henning et al., 2010; Kim et al., 2009; Songserm et al., 2006). Domestic ducks are often farmed in open fields, flooded rice paddies, or on ponds or other bodies of water. In countries with huge domestic duck populations, such as China, Vietnam and Indonesia, duck farming is closely integrated with rice production where ducks consume unharvested grain and fertilize fields as part of the scavenging process that ultimately provides both increased food and income for the farmer (James et al., 2008). Domestic ducks on smallholder farms are allowed to scavenge freely for food during the day around houses, in the villages or on rice paddies, with duck owners supplying little or no feed (Chen, 2009). This type of farming practice offers little opportunity for biosecurity and allows direct exposure to other village poultry and to wild birds that may introduce or spread virus to other farms. Duck flocks may also be moved long distances through a region as part of the scavenging process or on the way to markets which provides opportunities for further dissemination of H5N1 HPAI viruses (Henning et al., 2010, 2013).

Considering the widespread infection of waterfowl with H5N1 HPAI viruses in certain parts of the world, reducing the risk of virus infection in domestic ducks is essential for controlling the spread of the H5N1 HPAI (Beato et al., 2007; Chen et al., 2004; Hulse-Post et al., 2005; Li et al., 2004; Swayne, 2006). Biosecurity is a critical tool in the control H5N1 HPAI and can help prevent the spread of influenza viruses from wild to domestic ducks and vice versa; however, it is not easy to implement in certain types of farming (Beato et al., 2007). Ducks raised in closed high-biosecurity systems in Thailand were shown to be protected from infection in locations where H5N1 HPAI viruses were actively circulating among backyard ducks, open house ducks, and grazing ducks (Songserm et al., 2006). Live poultry markets (wet markets) have also been identified as an epidemiologic risk factor of infection (Webster, 2004). In the Hong Kong wet markets, the ban on ducks, geese, and later, quail, together with improved biosecurity (clean days), markedly reduced the influenza virus diversity (Peiris et al., 2007). In China, the predominance of live-bird marketing and the lack of segregation of

species probably increased the chance of spread of H5N1 HPAI viruses to other poultry and humans. This has been reduced to some extent through the use of vaccination and through segregation of terrestrial and aquatic birds in markets and during transportation (FAO, 2013).

The pathogenesis of AI virus infections in ducks is dependent on the strain of virus. The natural occurring low pathogenic endemic viruses are typically enterotropic with shedding primarily through feces. However, when waterfowl viruses jump to and become adapted in gallinaceous species, the virus typically changes to be more respiratory-tropic with much smaller amounts of detectable virus in feces. When these "chicken" adapted viruses infect ducks, the virus typically retains the respiratory-tropic replication pattern (Guo et al., 2000; Spackman et al., 2010). The Asian lineage H5N1 virus is a "chicken" adapted virus, and infected ducks have high virus replication in the lungs and upper respiratory tract. However, the method of transmission between ducks and from ducks to other species is not clearly understood. Because of the typical aquatic environment, virus shed in feces, oral secretions, and even from feather follicles, can all contribute to contamination of the environment or direct infection. Studies under sterile laboratory conditions have shown that AI viruses can remain infective in water for long periods of time (up to several months at 17 °C), but persistence varies depending on the virus and the chemical and physical conditions of the water (pH, salinity, and temperature) (Brown et al., 2007; Lebarbenchon et al., 2011; Stallknecht et al., 1990a, 1990b). The duration of infection and virus shedding on a flock basis in ducks under field conditions is not known and will depend on the rate of introduction of susceptible birds to the flock and the duration of immunity in individual ducks following natural exposure (Sims, 2012).

2. Use of vaccination to control H5N1 HPAI in domestic ducks

When biosecurity measures are impractical to enforce, vaccination is one of the few control tools available to help protect ducks against H5N1 HPAI. However, many factors are involved in the effective immunization of poultry for avian influenza, and relatively little information is available on vaccination in domestic duck species. In countries with large duck populations and persistent H5N1 HPAI, vaccines are being used as a tool in control programs in domestic ducks as well as other commercial and backyard poultry. On a flock basis, vaccination can help break the infectious cycle in combination with vigilant monitoring and strong biosecurity measures (Swayne, 2003), and has been shown to increase resistance to field challenge and to reduce virus shedding (Ellis et al., 2004; Swayne et al., 2001, 2006). Many studies done in domestic ducks indicate that vaccination can be successful in preventing clinical disease and reducing virus shedding (see Section 2.3), but the extent and quality of the immune response depends on the vaccine and vaccination strategy used.

In the field, the majority of the vaccines used in domestic ducks have been oil-emulsion inactivated vaccines, produced using low pathogenic avian influenza viruses that are naturally occurring or are produced by reverse genetics. A complete review of the vaccines used to protect poultry against H5N1 HPAI and information on the usage of H5N1 vaccines in ducks in different countries can be found elsewhere (Spackman and Swayne, 2013; Swayne et al., 2011). Vaccination of ducks and other poultry is practiced in small commercial farms, villages and households, but flock health and vaccination records are poor. Therefore, improved surveillance systems are needed to determine the immune status of vaccinated duck flocks and to ensure that H5N1 HPAI viruses do not continue to circulate within flocks. The difficulty of adequately vaccinating

sufficient number of ducks to maintain “herd immunity” is a huge obstacle in the control of H5N1 HPAI. The logistics of vaccinating ducks when they are not confined in a biosecure location compounds the problem because individual ducks are not uniquely identified. The countries with large duck populations are generally poor and with weak public infrastructure, so the inability to get enough high quality vaccine, maintain the cold chain, and have skilled veterinary technicians to administer the vaccine are also roadblocks to successful vaccination programs. In China, where up to four billion ducks are reared annually, often in open fields with no biosecurity measures, vaccination coverage against H5N1 HPAI is poor, and therefore huge numbers of ducks remain susceptible and serve as reservoirs and disseminators of H5N1 HPAI viruses (Qian et al., 2012). Layer and breeder ducks are vaccinated when they are young, but adult ducks do not receive the required booster vaccination. Waterfowl raised for meat consumption are usually not vaccinated because of their short lifespan. Consequently practical implementation of vaccination may be impaired because vaccination coverage may never reach all of the susceptible birds (Chen, 2009).

In addition, H5N1 HPAI viruses continue to undergo antigenic drift while circulating in nature. Thus, vaccines need to be updated to match the circulating and emerging strains of H5N1 HPAI viruses. Continuous new outbreaks emphasize the need for a comprehensive waterfowl vaccination strategy and the development of waterfowl-specific efficient vaccines (Peyre et al., 2009).

2.1. Determining vaccine efficacy in ducks

In a successful vaccination program, the vaccine must protect the vaccinated animals against clinical signs of the disease and prevent mortality; reduce virus shedding into the environment; and increase the minimum dose of virus required to infect a bird, therefore limiting contact infection and spread of the disease (Peyre et al., 2009). Immunological principles for AI vaccine protection have been reviewed elsewhere (Suarez and Schultz-Cherry, 2000; Swayne and Kapczynski, 2008). Protection against AI is mostly conferred by the production of antibodies against the hemagglutinin (HA) viral protein. Therefore, the level of seroconversion and titers in terms of anti-HA antibodies, measured by the hemagglutination inhibition (HI) test, is the most commonly used method to evaluate vaccine efficacy (OIE, 2012). Although differences in HI titers can be observed between vaccines and bird species, most vaccines confer high levels of protection against clinical signs and mortality (Peyre et al., 2009). However, the association between serological titers and protection against viral challenge is not so clear for vaccines in ducks. For example, ducks vaccinated with low antigen doses of a recombinant vaccine did not produce detectable HI antibody titers but were fully protected against a lethal challenge with a H5N1 HPAI virus (Webster et al., 2006), indicating that titers above a certain threshold are predictive of protection, but are not predictive below this threshold (Spackman and Swaine, 2013).

An additional concern is the limited protection that can occur with vaccines heterologous to the challenge virus. Most commercial AI vaccines are able to confer 100% protection against experimental challenge with homologous viruses and offer some level of cross-protection against heterologous strains of the same subtype. Nevertheless, discrepancies in viral shedding (cloacal and tracheal) have been linked to genetic differences between the vaccine and challenge virus strains (Peyre et al., 2009). Studies in chickens have demonstrated a direct correlation between the HA sequence similarity of the vaccine and challenge viruses and the ability of the vaccine to reduce tracheal shedding (Swayne et al., 2000). Similar results were seen in ducks and geese when using a single dose of killed oil adjuvanted vaccine, where the homologous vaccine

significantly reduced virus shedding compared to the heterologous vaccines (Eggert and Swaine, 2010; Pfeiffer et al., 2010). However, differences in immune response to AI vaccination have been reported between chickens and ducks (Magor, 2011). Vaccination of ducks with reverse-genetics engineered inactivated oil emulsion vaccine, while efficacious, required larger doses and a second boost of vaccine in comparison to chickens (Tian et al., 2005). Vaccination comparisons of chickens and ducks with an inactivated oil emulsion vaccine show chickens have a much more robust antibody response to the vaccine than naïve ducks (Webster et al., 2006). Although the ducks were protected from death and disease signs, the challenge H5N1 virus did replicate in the vaccinated ducks. The reasons for the weaker immune response of ducks are not clear, but immunoglobulin structure and function might provide some genetic clues to their distinct humoral immune response (Magor, 2011).

Evaluation of vaccine efficacy differs greatly from the laboratory to the field setting, with laboratory vaccination studies often providing excellent protection that contrasts with poor protection in the field. These differences can be attributed to many causes including variations in the immune status of the vaccinated birds (immunosuppressive conditions and concurrent diseases), maternal immunity, genetic factors, husbandry conditions (environmental stress), and technical issues related to vaccine administration in the field. Limited field trial data is available so far and international and/or national authorities should ensure that new vaccine formulations are being validated in field pilot studies prior to routine use in mass vaccination campaigns (Peyre et al., 2009). Almost no field studies are performed on efficacy of vaccination in ducks, with the expectation that studies performed in chickens will translate to other species. However, that assumption is likely incorrect because of differences in the immune responses between these two species. The importance of using vaccination protocols specifically designed for domestic ducks was demonstrated during a vaccination survey in northern Vietnam, in which the need to increase vaccination doses to induce protective immune response in this species was shown (Capua and Cattoli, 2013; Desvaux et al., 2012b).

2.2. Evaluation of vaccine performance in the field

2.2.1. Post-vaccination surveillance

To improve control of H5N1 HPAI, vaccination programs should incorporate post-vaccination surveillance strategies to determine the effectiveness of the vaccination program (Capua and Marangon, 2006). Vaccine efficacy and virus circulation within vaccinated flocks should be monitored as recommended by international authorities by using virological and immunological methods (Peyre et al., 2009). One of the biggest problems with AI vaccination is that a virus might circulate undetected, which increases the risk of subsequent outbreaks and/or antigenic drift of the circulating virus away from the vaccine strain. At the flock level, a simple method to identify infected flocks consists of regularly monitoring sentinel birds left unvaccinated in each vaccinated flock, but this approach does have some management problems, particularly with regards to identifying the sentinels in large flocks (OIE, 2012). As an alternative or adjunct system, testing for field exposure may be performed on the vaccinated birds either by detection of field virus or antibodies against the virus. To detect the field virus, oropharyngeal or cloacal swabs from baseline daily mortality or sick birds can be tested, individually or as pools, by molecular methods, such as real time reverse transcription polymerase chain reaction (rRT-PCR) or antigen capture enzyme-linked immunosorbent assay (ACELISA) of the vaccinated populations (Swayne and Kapczynski, 2008).

When using commonly available diagnostic tests to conduct serosurveillance, as for example in China, Vietnam and Indonesia,

where duck flocks are being vaccinated with killed H5N1 vaccines, vaccinated and naturally infected birds will both produce H5-specific antibody, complicating the interpretation of the tests. Domestic ducks can also be infected sub-clinically with other circulating AI viruses, further complicating serosurveillance. Various strategies have been developed for differentiating infected from vaccinated animals (DIVA) (Suarez, 2012). Although several DIVA strategies based on the specificity of antibody responses have been devised, they all have serious limitations, especially for testing ducks. For example in Asia, ducks are currently being vaccinated with reverse genetics-derived killed H5N1 vaccines that do not allow the use of the heterologous neuraminidase (NA) type of DIVA strategy. Circulation of other AI viruses in these countries will generate nucleoprotein (NP)-specific and non structural (NS)1-specific antibody responses that complicate use of NP- and NS1-specific tests to monitor H5N1 infection (James et al., 2008). Also any circulating HxN1 viruses (e.g. H6N1, H7N1) will impede the use of N1 antibody testing if a heterologous NA vaccination DIVA strategy is in place. At present the NS1-specific tests have not been fully validated for use in an AI monitoring system and the heterologous NA DIVA approach has only been used for low pathogenicity AI virus surveillance in chickens and turkeys (Grogan et al., 2007).

A study evaluating the performances of the HI test and of a H5-ELISA using samples from chickens and ducks in Vietnam, showed that performance of the HI test was very good, supporting the cut-off of $>4 \log_2$ used for the HI test for chickens but suggesting that a $>3 \log_2$ cut-off was more appropriate for ducks (Desvaux et al., 2012a). When compared with the virus neutralization test, the H5-ELISA showed poor specificity when using the positive cut-off specified by the manufacturer, but could be used as a screening test if confirmed by the HI test or a virus neutralization test. A general and highly sensitive pre-screening test for the detection of NP-specific antibodies with a competitive ELISA can be used to identify positive birds, but this test needs to be confirmed by HI. This approach appears of little value in the context where ducks are exposed to a number of different influenza viruses when only a single subtype is targeted for surveillance and control. Therefore serologic surveillance must be supplemented with virus detection and characterization programs to understand virus persistence in a country or region (Desvaux et al., 2012a). However, there remains a large funding gap in most endemic countries to perform proper surveillance that is unlikely to be supported by international donors because of donor fatigue after many years of support (Sims, 2012).

In a study examining farm and flock-level risk factors associated with H5N1 HPAI outbreaks on small holder duck and chicken farms in the Mekong Delta of Vietnam, it was found that the odds of having an outbreak were highest in unvaccinated flocks, intermediate in flocks vaccinated once, and lowest in flocks vaccinated twice (Henning et al., 2009). Mathematical models suggest that 90% of a flock needs to be vaccinated to reduce the probability of an outbreak by 50% (Savill et al., 2006) yet in field situations, commonly less than 90% of birds in a flock are vaccinated (Suarez, 2005). In this study, no farms had full vaccination coverage (two vaccinations 4 weeks apart) in all of their flocks. Results of this study indicated that across all flocks, scavenging contributes to a relatively small increase of risk of H5N1 HPAI outbreaks when compared to the increase in risk due to non-vaccination. However, amongst scavenging flocks, those that share scavenging locations with ducks from other farms are at increased risk of an H5N1 HPAI outbreak. This suggests that a stricter approach to biosecurity within the practice of scavenging could decrease the risk of avian influenza without banning the practice altogether. The authors conclude that none or only single vaccination, visits by family and friends to farms, the presence of geese on farms and sharing of scavenging areas with ducks from other farms increase the risk of H5N1 HPAI outbreaks in poultry flocks.

2.2.2. Vaccine effectiveness: Quality of protection and vaccination programs

Not many studies have been conducted evaluating the efficacy of duck vaccination in the field. A study examining antibody kinetics induced by vaccination, found that ducks developed HI antibodies quickly after vaccination, reaching $8 \log_2$ titers 4 weeks post-vaccination and gradually declining to $4 \log_2$ by 14 weeks post-vaccination (Tian et al., 2005). The antibody titers increased rapidly to $10 \log_2$ 1 week after a second vaccine dose was given and remained at $6 \log_2$ for 38 weeks. Ducks challenged at this time point with a H5N1 HPAI virus were protected from clinical disease, although low titers of virus were detected from oropharyngeal swabs of some ducks three days after challenge. It is not clear if these results can be extrapolated to other vaccines and vaccination protocols.

A study conducted in Vietnam to evaluate virus transmission within infected flocks before and after vaccination found that results were consistent with vaccine trials that suggest that the use of vaccines is expected to reduce virus transmission and associated mortality among poultry by increasing the incubation period and reducing virus shedding (Soares Magalhaes et al., 2010). Apart from issues related to the quality of protection provided by the vaccine, the overall effectiveness of vaccination campaigns was shown to be undermined by factors that deter farmers with commercial size flocks from vaccinating their flocks, and operational issues for vaccine delivery. The former may be linked to the length of vaccine-withholding period and rumors concerning adverse reactions to the vaccine while the latter may be affected by issues such as training and payment of vaccinators, spoilage of vaccine stocks and rapid turn-over of at-risk populations (Soares Magalhaes et al., 2010). The authors suggest that if vaccination continues to be included as part of a sustainable disease control program, efforts should be focused on training farmers in disease prevention in addition to disease recognition since the second is likely to be compromised in a vaccinated population. Efforts must also be made to reduce operational delays in the implementation of disease control interventions after the recognition of the initial outbreak.

Another study was undertaken to understand the epidemiology of H5N1 HPAI in the context of vaccination and to draw some conclusions about vaccination efficacy in the domestic poultry population of the Red River Delta area in Vietnam (Desvaux et al., 2012b). Five cross-sectional surveys to measure the serological and virological prevalence in vaccinated and unvaccinated poultry were performed. The size of the flock was found to be a determinant of seroconversion probability with a higher risk of not seroconverting for the smaller size flocks, probably because of the greater technical capacity of farmers in bigger farms leading to less frequent preventable vaccine failures. However, birds from large flocks had a mean HI titer lower than birds from small flocks, most likely because intensive management practices for the largest flock induces more stress for the birds, and as a consequence, lowers the immunological response. An effect of the vaccination protocol on the HI titer of seroconverted ducks was also found, with a lower mean HI titer for birds vaccinated once at more than 20 days of age, than for those vaccinated twice. This study also highlighted the difficulties in maintaining good flock immunity in poultry populations using inactivated vaccine in the field with two vaccination rounds per year, and in preventing circulation of virus in co-existing non-vaccinated poultry (Desvaux et al., 2012b). The seroprevalence measured by the presence of HI H5 antibodies in the studied population was <30% for all the sampling campaigns. Serological and virological results indicate that the vaccination levels being achieved did not prevent circulation of virus in co-existing unvaccinated poultry. Several factors were suggested to contribute and explain this low immunity level including: The high population turnover in poultry production systems that does not allow

the vaccination of all birds with a bi-annual vaccination campaign; different causes of preventable failures such as problems with the cold chain that could have a direct consequence on the effect of the vaccine, incorrect injection techniques, incorrect dosages that could lead to birds not receiving the appropriate amount of antigen; and inappropriate vaccination protocols leading to low or no immunological response (Desvaux et al., 2012b). The importance for vaccine immunogenicity of the number of doses and age of the ducks at the time of vaccination was also confirmed in this study, which correlates with reports on the need to increase vaccine doses to induce protective immune response in ducks (Kim et al., 2008). A fall in antibody levels at 1–2 months post-vaccination was observed and might also be explained by inappropriate vaccination protocols used in the field. Despite failure in stopping virus circulation, an indirect protection of unvaccinated birds was shown when the vaccinated population of the same commune showed seroprevalence levels between 50% and 70% compared to situations where this seroprevalence level was <50%. Also, the role played by ducks in maintenance of the virus was demonstrated as the probability of seroconversion of unvaccinated birds was higher in June, when the meat-duck population reaches its maximum size. Meat ducks most likely contribute to virus dissemination because of their farming management, as they are allowed to scavenge all day in the rice fields.

2.2.3. Duration of immunity in vaccinated ducks

In laboratory studies, vaccination against H5N1 influenza viruses has protected ducks against lethal challenge, but the duration of the antibody response, which is a crucial factor in the field, has not been fully addressed. As mentioned previously, Tian et al. detected high antibody titers at 38 weeks in ducks vaccinated twice with a H5N1 inactivated vaccine developed by reverse genetics (Tian et al., 2005). In a study by Boltz et al. (2009) H5N3 HI and virus neutralization antibodies were observed 40 weeks after vaccination of ducks with one dose of an inactivated reverse genetics-derived H5N3 vaccine. This study demonstrated that the inactivated vaccine produced long-lasting antibodies to homologous and heterologous viruses under field conditions as cross-clade antibodies to a H5N1 virus (A/chicken/Laos/A0464/07), a virus antigenically distinct from the vaccine strain, were also detected after a single vaccination and were sustained for 28 weeks (for 40 weeks when a boost vaccination was given). In another study, the duration of humoral immunity in ducks was evaluated by testing serum samples from ducks vaccinated with a whole virus inactivated oil emulsion vaccine (Beato et al., 2007). The log₂ geometric mean titer (GMT) of the vaccinated ducks after the first vaccination was 2.69 and increased to 7.69 after a second vaccination. The antibody titers remained at acceptable levels through 20 weeks with log₂ GMT 4, but by week 28 the log₂ GMT was 2.46, below the value considered to be consistently protective. The results of these studies emphasize the need for more research on duration of antibody responses elicited by vaccination in ducks, since duration of immunity can vary depending on the vaccine, dose, vaccination protocol and many other factors.

2.2.4. Virus recovery from tissues of vaccinated-challenged ducks

There has been concerns about the presence of virus in duck meat, both for the implications on disease transmission to animals through swill feeding (Harder et al., 2009; Mase et al., 2005; Swayne and Beck, 2005), and for the food safety implications for humans (Beato et al., 2007). Viable virus has been recovered from duck meat and internal organs of ducks experimentally infected with H5N1 HPAI viruses (Pantin-Jackwood and Swayne, 2007; Tumpey et al., 2002), even though the ducks did not always show clinical signs. Studies in chickens have shown that vaccination can prevent virus replication in meat (Swayne and Beck, 2005). Likewise, no virus was

isolated from meat from vaccinated ducks challenged with a H5N1 HPAI virus (Beato et al., 2007). Similar results were obtained from the liver, which is an organ that is used to make food preparations such as *foie gras* in some countries of the world. Although virus levels of meat in the control birds were low, it was concluded that vaccination prevented the establishment of viremia and therefore prevented viral colonization of internal organs, positively influencing the food security of duck products (Beato et al., 2007). Vaccine studies using H5N1 HPAI viruses of different pathogenicity need to be done to corroborate these results.

2.3. Experimental studies evaluating vaccine efficacy in ducks

Several studies in laboratory settings have been conducted to evaluate vaccine efficacy in ducks against H5N1 HPAI virus challenge (Beato et al., 2007; Boltz et al., 2009; Cagle et al., 2011, 2012; Chua et al., 2010; Eggert and Swayne, 2010; Ferreira et al., 2012; Kim et al., 2008; Liu et al., 2011a; Middleton et al., 2007; Pfeiffer et al., 2010; Qian et al., 2012; Rudolf et al., 2009; Steensels et al., 2007, 2009; Tian et al., 2005; van der Goot et al., 2007, 2008; Webster et al., 2006; Yao et al., 2010). Details of these studies are presented in Table 1. These studies differed in many aspects including type of vaccine, duck species, vaccination protocol (age of the ducks at vaccination, number of vaccine doses, amount of HA antigen in vaccine), challenge virus, and experimental settings. Nevertheless, important information can be drawn from them. The findings reported in these studies have significant implications on practical aspects of H5N1 HPAI control. Based on the results of these studies, it appears that vaccination in most cases will protect ducks against disease and will reduce virus shedding following virus challenge. If the vaccine is a good match to circulating viruses and it is given correctly, vaccinated flocks should not shed enough virus to infect either vaccinated or unvaccinated birds. Several of the variables affecting vaccine efficacy are discussed below.

2.3.1. Types of vaccines and vaccination protocols

Most of the vaccines used in these efficacy studies are described in a separate review on vaccination of gallinaceous poultry for H5N1 HPAI (Spackman and Swayne, 2013). In the field, the majority of the vaccines used in domestic ducks have been oil-emulsion inactivated vaccines, produced or not by reverse genetics. Both commercially available and experimental vaccines have been evaluated in ducks (Table 1).

An inactivated vaccine produced with a strain generated by reverse genetics (Re-1), containing the NA gene and a modified HA gene of A/goose/Guangdong/1/96 (H5N1) in combination with the six other genes from A/PR/8/34 (H1N1) as backbone, was able to protect against clinical signs, including mortality, and it also reduced the viral load in ducks (Tian et al., 2005). This same vaccine, which was made commercially available, was used in three other studies (Cagle et al., 2011, 2012; Pfeiffer et al., 2010) showing variation in protecting ducks against lethal infection with different H5N1 HPAI viruses. The Re-1 vaccine induced low to moderate antibody titers in young Pekin (*Anas platyrhynchos var. domestica*) and Muscovy ducks (*Cairina moschata*), protecting them against disease after challenged 2 weeks after vaccination, but not preventing virus shedding. The results were affected by duck species, vaccine schedule, and challenge virus used. Likewise, one administration of a conventional inactivated vaccine was shown to protect Pekin ducks from mortality 1 week post-vaccination, but clinical signs caused by H5N1 infection were only reduced and virus was transmitted to contact ducks (van der Goot et al., 2007). Administration of one of four different whole virus inactivated vaccines to 1 week-old Pekin ducks did not eliminate virus shedding, with reduction only observed in ducks vaccinated with the vaccine homologous to the challenge virus (Eggert and Swayne, 2010). Results of these

Table 1
Studies on efficacy of H5 influenza vaccines against H5N1 HPAI virus challenge in domestic ducks.

Vaccine	Type of ducks	Vaccination protocol*	Mean HI titers (\log_2)	Virus challenge	Clinical signs	Viral shedding	Ref.
Recombinant inactivated H5N1 vaccine-Re-1. HA and NA from A/Gs/Guangdong/1/96 (HA clade 0)	Sheldrake	3-wks. IM; 0.5 ml containing 4.6 μ g of HA antigen	–	6 wks. with $10^{7.5}$ EID ₅₀ of DKSH/04 (HA homology with vaccine virus is 94.6%)	No clinical signs in vaccinated ducks. 13/15 non-vaccinated controls died	No virus shedding in vaccinated ducks. All non-vaccinated controls shed virus at 3 and 5 days post challenge	Tian et al. (2005)
Recombinant inactivated oil emulsion vaccine H5N3 (with modified H5 from A/Ck/Vietnam/C58/04)	Pekin	One or two doses containing 0.015 to 1.2 μ g of HA protein. At 2 wks. (single) or at 2 and 5 wks. (double). IM	>7	8 wks. Single: IN with 100 duck lethal doses of A/duck/Thailand/71.1/04 Double: IN 30 CLD ₅₀ of A/Ck/Vietnam/C58/04 IN/intratracheal with 10^4 EID ₅₀ of A/duck/Thailand/71.1/04	100% protection against mortality	No or minimal virus shedding in vaccinated ducks challenged with either virus. Most non-vaccinated control ducks shed virus	Webster et al. (2006)
	Khaki Campbell	0.25 μ g of HA 2 wks. (single) or 2 and 4 wks. (double)	>9	IN/intratracheal with 10^4 EID ₅₀ of A/duck/Thailand/71.1/04			
Whole virus inactivated oil emulsion vaccine: Bivalent Poulevac i-AI H5N9, H7N1 (Fort Dodge); and monovalent Poulevac i-AI H5N3 RG (modified HA from A/Ck/Vietnam/C58/04)	Pekin	1-day-old and 3 wks. SQ. 0.2 ml	2–3 with bivalent vaccine. 3–6 with monovalent vaccine 3 wks p.v. *used challenge virus in test	6 wks. with $10^{1.5}$ DID ₅₀ IN, orally and intraocular of A/Muscovy duck/Vietnam/453/04	0/0/15 (morbidity/mortality/total) in vaccinated ducks compared to 14/9/14 in non-vaccinated controls	0–2/14 of vaccinated ducks shedding compared to 14/14 of non-vaccinated ducks	Middleton et al. (2007)
Whole virus inactivated oil emulsion vaccine containing the low pathogenicity avian influenza (LPAI) strain A/duck/Potsdam/1402/86 (H5N2)	Pekin	1-day-old and 4 wks. SQ	7.69 at challenge >4 up to 4 months p.v.	9 wks. IN and oral with $10^{7.0}$ EID ₅₀ of A/duck/Vietnam/12/05	0/0/10 (morbidity/mortality/total) in vaccinated ducks compared to 10/7/10 in non-vaccinated controls	0/10 of vaccinated ducks shedding compared to 9/10 of non-vaccinated ducks	Beato et al. (2007)
Whole virus inactivated vaccine (H5N9-It); vectored vaccine (vFP-AIV-H5)	Muscovy	Different doses, SQ. Group1: H5N9-It, 5 and 7 wks. (0.5 and 1.0 ml respectively) Group 2: rFP-AIV-H5, 5 and 7 wks.-of age, 100 chicken dose	7.5 (H5N9-It) 3 (rFP-AIV-H5) 13 days p.v.	9 wks. Oculo-nasal route. 10^7 EID ₅₀ of A/crested eagle/Belgium/01/04	0/0/10 (morbidity/mortality/total) in vaccinated ducks compared to 10/7/10 in non-vaccinated controls	Number of vaccinated ducks shedding: Group 1: 9/10; Group 2: 7/10. Reduced virus amounts compared to non-vaccinated ducks	Steensels et al. (2007)

Table 1 (Continued)

						G Model VIRUS-96034; No. of Pages 14	
Whole virus inactivated oil emulsion vaccine based on A/Ck/Mexico/232/94/CPA H5N2 (Intervet Schering-Plough, The Netherlands)	Pekin	7-wks., single vaccination; 6 wks., single vaccination; 6 and 9 wks., double vaccination SQ, 0.5 ml	7, double vaccination. No or low HI titers with single vaccination. 2 wks. p.v.	8 wks. for single vaccination and 11 wks. for double. $10^{5.3-5.8}$ EID ₅₀ of A/Ck/China/1204/04 H5N1 (HA clade 2.4) IN and intratracheally. (HA1 homology between vaccine and challenge virus: 84%)	2–3/0/10 (morbidity/mortality/total) in vaccinated ducks compared to 8/2/10 in non-vaccinated controls	9–10/10 ducks given single vaccination. 4/10 in double vaccinated ducks. 10/10 non-vaccinated controls shed virus	van der Goot et al. (2007, 2008)
Three recombinant inactivated oil emulsion whole-virus H5 influenza vaccines made from H5N1 viruses (HA clades 1, 2, 2 and 2.3.4)	Pekin	2 wks., IM, single dose, 1 µg of HA	14–20 GMT. 3 wks. p.v.	5 wks. IN, intraocular, and intratracheal with $10^{1.25}$ EID ₅₀ of Dk/Laos/25/06 (HA clade 2.3.4)	0/0/10 (morbidity/mortality/total) in vaccinated ducks compared to 9/9/10 in non-vaccinated controls	0/10 ducks for all vaccinated groups	Kim et al. (2008)
Whole virus inactivated vaccine (H5N9-It); and/or vectored vaccine (vFP-AIV-H5) expressing the HA gene of A/Ck/Indonesia/7/03	Pekin	17 and 42 days. SQ; vaccinated once or twice with H5N9-It (0.5 ml single or 1 ml double chicken dose) or with the vFP-AIV-H5 vaccine (10^5 TCID ₅₀ /dose)	6 or 9.5 in H5N9It vaccinated ducks (1× or 2×) 6 in vFP-AIV-H5 × 2 vaccinated ducks 9 in vFPH5–H5N9-It vaccinated ducks	56 days with 10^7 EID ₅₀ of A/crested eagle/Belgium/01/2004 (HA clade 1)	0/0/10 (morbidity/mortality/total) in vaccinated ducks compared to 2/2/10 in non-vaccinated controls	None of the vaccinated-challenged ducks shed virus. 90% of the controls did	Steensels et al. (2009)
Inactivated and adjuvanted vaccine based on LPAI strain A/duck/Postdam/1402/86 (H5N2)	Pekin	7 and 11 wks. IM. 0.1 ml	≥5 at 14 wks.	10^6 EID ₅₀ of A/Cygnus Cygnus/Germany/R65/06 (H5N1) at 10, 14 or 26 wks. of age; oculo/nasal route 3 wks. IN with $10^{5.0}$ EID ₅₀ of Dk/VN/203/05 (HA clade 2.3.2) or Dk/VN/218/05 (HA clade 2.3.4)	Non-vaccinated and vaccinated ducks displayed no clinical signs	Low number of non-vaccinated and vaccinated ducks shed virus	Rudolf et al. (2009)
Whole virus inactivated commercial vaccines: Tk/England/N-28/73 (N28); Re-1 (Harbin Veterinary Res. Institute); and A/Ck/Mexico/232/94 (H5N2) (Intervet International)	Pekin	1wk. SQ, 0.3 to 0.5 ml of vaccine	4–6	10^6 EID ₅₀ of Dk/VN/203/05 (HA clade 2.3.2) or Dk/VN/218/05 (HA clade 2.3.4)	0/0/10 to 1/1/10 (morbidity/mortality/total) in vaccinated ducks compared to 10/10/10 in non-vaccinated controls	More than 30% of vaccinated ducks in all groups shed virus at 3 days post challenge	Pfeiffer et al. (2010)
DNA vaccine with HA from A/mallard/BC/373/2005 (H5N2 LPAI). Vector controls (pSec); ptHA; and ptHA-tCD154	Pekin	Exp.1: 3–4 wks. immunized on wks. 0, 4, 8, 12. Exp. 2: 2–7 days immunized on wks. 0, 2, 4, 6, and 11. 200 µg of plasmid IM	Exp.1: 0.5 ptHA 4 ptHA-tCD154 (wk 9) Exp. 2: 5.5 ptHA-tCD154 (wk 8)	3 and 22 wks. after last immunization (exp.1) and at 3 wks. (exp.2) with A/Ck/Vietnam/14/2005 10^5 PFU. Intraocular, IN and oral	Only one of the controls was euthanized. Immunization with ptHA; and ptHA-tCD154 delayed and mitigated clinical signs	All vaccinated ducks shed virus	Yao et al. (2010)

Table 1 (Continued)

Vaccine	Type of ducks	Vaccination protocol ^a	Mean HI titers (\log_2)	Virus challenge	Clinical signs	Viral shedding	Ref.
Subunit H5 vaccine. Recombinant baculovirus expressed H5 vaccine (TLL/H5N2); inactivated whole virus vaccine with tetanus toxoid marker antigen (TT/H5N2); commercially available inactivated H5N2 whole virus vaccine (Intervet International)	Pekin hybrid	1–4 wks. SQ. Additional dose of TLL given at 5 wks. The TT/H5N2 and the H5N2 vaccine dose was 1.0 ml (twice the dose given to chickens)	16 GMT for TLL/H5N2 119 GMT for inac.H5N2 49 GMT for TT/H5N2 vaccinated ducks	9 wks. with $10^{5.3}$ EID ₅₀ eye drop/IN/orally A/Vietnam/1203/2004 (HA clade 1)	Morbidity/mortality/total) in vaccinated ducks: TLL/H5N2 0/0/8 Inac. H5N2v 0/1/7 TT/H5N2 0/1/7 Controls 2/5/7	No virus shedding in vaccinated ducks when examined at 2 and 4 dpi. 7/7 of controls shed virus	Chua et al. (2010)
Whole virus inactivated oil emulsion vaccines: A/turkey/WI/1968 (H5N9); A/Ck/Hidalgo/28159-232/1995 (H5N2); A/tk/England/N28/1973 (H5N2); and A/ck/Indonesia/7/2003 (H5N1)	Pekin	7-day-old. SQ. Dose: 0.5 ml	12–16 GMT 3 wks. p.v.	3 wks. post vaccination with 10^6 EID ₅₀ A/Ck/Indonesia/7/2003 (HA clade 2.1). IN	0/0/6 (morbidity/mortality/total) in vaccinated ducks 0/0/6 in non-vaccinated controls	3–6/6 vaccinated ducks shed virus. Reduction in shedding was only observed in ducks vaccinated with the homologous virus (A/Ck/Indonesia/7/2003)	Eggert and Swayne (2010)
Recombinant inactivated Re-1 vaccine	Pekin and Muscovy	SQ at 1 and at 14 days; at 14 days; or at 7 and 21 days. 0.2–0.5 ml	5–7 in Pekin ducks 3–7 in Muscovy ducks, depending on vaccine schedule	At 30 days with $10^{5.0}$ EID ₅₀ of A/Dk/Nam Dinh(VietNam)/NCVD-88/2007 (HA clade 2.3.4)	Vaccinated ducks: 0–5/8 presented clinical signs and 0–3/8 died Non-vaccinated controls: 6/6/6 (morbidity/mortality/total) Best protection when vaccinated at 7 and 21 days of age. Higher morbidity and mortality in Muscovy ducks than Pekin	All Muscovy ducks shed virus compared to 1 to 4 Pekin ducks per group at 3 days after challenge	Cagle et al. (2011)
Virus-vectorized bivalent vaccine. Live attenuated duck enteritis virus (DEV) expressing the HA gene of A/duck/Anhui/1/06 H5N1(HA clade 2.3.4)	Unknown	4 wks., or 4 and 7 wks. one or two doses. IM, 0.1 ml	>3 single high dose at 3 wks. p.v. >5 two doses, 1 wk. p.v.	100-fold DLD ₅₀ of HB/49 (HA clade 2.3.4) at different time points; 3 days, 1 wk, 3 wks., 10 wks. IN	All non-vaccinated ducks died. 5–8/8 vaccinated ducks survived	Minimal or no virus shedding in vaccinated ducks	Liu et al. (2011b)

Table 1 (Continued)

Recombinant fowlpox virus vectored vaccine coexpressing the HA gene from A/mallard/Huadong/SY/2005 (H5N1) and the chicken interleukin 6 gene(rFPV-AI-AIL6); rFPV expressing the HA gene alone (rFPV-SYHA; Re-5 recombinant inactivated vaccine (HA clade 2.3.4)	Gaoyou and Cherry Valley ducks	Gaoyou 24 days, Cherry Valley 10 days. SQ; 10^5 PFU of rFPV-AI-AIL6 and rFPV-SYHA; 0.5 ml of Re-5	1.17–2.57, rFPV-SYHA and rFPV-AI-AIL6 > 4, Re-5 at 3 wks. p.v.	21–23 days p.v. with 0.2 ml of $10^{5.75}$ EID ₅₀ of A/mallard/Huadong/SY/2005 in nares and by eye drop	Gaoyou: rFPV-AI-AIL6 and Re-5 80–100% of ducks survived; rFPV-SYHA 41.7% of ducks survived Cherry Valley: rFPV-AI-AIL6 and Re-5, 90–100% of ducks survived; rFPV-SYHA 54.5% of ducks survived. No survivors in non-vaccinated control groups	Reduced virus shedding in ducks vaccinated with rFPV-AI-AIL6 compared to those vaccinated with rFPV-SYHA, and similar to shedding in Re-5 vaccinated ducks	Qian et al. (2012)
Recombinant inactivated Re-1 vaccine	Pekin and Muscovy	14 days. SQ; 0.5 ml	Pekin: 2.8 (5/10 ducks) Muscovy: <2 (2/10 ducks) 2 wks. p.v.	30 days, IN with $10^{5.0}$ EID ₅₀ of A/Dk/NauGiang/NCVD07-12/07 (HA clade 1)	Morbidity/mortality/total in vaccinated ducks: Pekin: 1/10 Muscovy: 4/4 In non-vaccinated: Pekin: 3/3 Muscovy: 6/6	Number of vaccinated ducks shedding at 3 days post challenge: Pekin: 2/10 Muscovy: 6/8	Cagle et al. (2012)
Virus-vectorized (NDV) vaccine expressing the HA gene from A/turkey/Turkey/1/2005 (H5N1)	Mule ducks	11 days and 2 wks. after. Ocular route, $10^{6.0}$ EID ₅₀ of the vaccine	>3 at 4 wks. after second vaccination (85% of vaccinated ducks)	2 wks. after second vaccination, IN and ocular with $10^{6.0}$ EID ₅₀ of A/duck/Hungary/1180/2006	Morbidity/mortality/total in vaccinated ducks: 0/0/10 In non-vaccinated: 2/2/10	No virus shedding in vaccinated ducks	Ferreira et al. (2012)

CLD₅₀ = 50% chicken lethal dose; DLD₅₀ = 50% duck lethal dose; EID₅₀ = 50% embryo infective dose; HA = hemagglutinin; HI = hemagglutination inhibition; IM = intramuscular; IN = intranasal; SQ = subcutaneous; PFU = plaque forming units; p.v. = post-vaccination; wks. = weeks.

* Age of ducks at vaccination, vaccine dose, and vaccination route.

studies indicate that a single vaccination dose is probably not sufficient to reduce transmission of H5N1 HPAI virus and suggests that a second vaccination administration is necessary to block transmission. The need for two vaccinations was confirmed by a second study by van der Goot et al. (2008) which showed that two administrations with the whole virus inactivated vaccine conferred better protection by reducing viral shedding in vaccinated ducks.

The efficacy of different vaccination protocols was also evaluated in 17-day-old Pekin ducks by using an experimental inactivated whole virus vaccine and/or a fowlpox recombinant expressing a synthetic HA gene from an Asian H5N1 isolate (Steensels et al., 2009). This study showed that full protection against clinical signs and shedding was induced by the different vaccination schemes. However, the broadest antibody response and the lowest antibody increase after challenge were observed in the group of ducks whose immune system was primed with the fowl pox vectored vaccine and boosted with the inactivated vaccine, suggesting that this prime-boost strategy induced optimal immunity against H5N1 and allowed only minimal viral replication after challenge in ducks. In addition, this prime-boost vaccination scheme was shown to be immunogenic in 1-day-old ducklings. This study and a previous one (Steensels et al., 2007) showed that despite the poor replication of fowl pox virus in ducks, fowl pox vectored vaccines are immunogenic and protective in this species after two administrations of approximately 100 times a chicken dose. Field trials in 1-day-old ducklings are needed to confirm induction of an earlier onset of immunity and of a broader cross-reactivity to various antigens, and to evaluate the duration of protection induced by such prime-boost vaccination scheme compared to two shots of inactivated vaccines in field conditions (Steensels et al., 2009).

2.3.2. Effect of species or breed of ducks in response to H5N1 HPAI vaccination

The majority of the published studies evaluating vaccine efficacy against H5N1 HPAI in ducks have been done using Pekin ducks (*A. platyrhynchos var. domestica*), and less research has been done been using Muscovy ducks (*C. moschata*), Sheldrakes (*Tadorna tadorna*), Gouyou ducks (Chinese indigenous species), Mule ducks (a hybrid between Muscovy males and Pekin females), or other type of *A. platyrhynchos* ducks (Cherry Valley, Khaki Campbell) (Table 1). Differences in response to vaccination between different types of ducks are difficult to assess when the experimental conditions are not the same. The two different vaccine efficacy studies using fowlpox-vectored AI, revealed differences between Pekin and Muscovy ducks in response to vaccination (Steensels et al., 2007, 2009). Oropharyngeal virus shedding occurred in vaccinated Muscovy ducks as late as 19 days post infection, while no shedding was detectable in vaccinated Pekin ducks at any point after infection with the same HPAI H5N1 virus. These differences between duck species in response to H5N1 vaccination were corroborated by other studies which clearly demonstrated a lower protection conferred by vaccination in Muscovy ducks to H5N1 HPAI virus infection when compared to Pekin ducks (Cagle et al., 2011, 2012). Vaccinated Muscovy ducks shed more virus and for longer periods of time than Pekin ducks, and had lower protection against morbidity and mortality (Cagle et al., 2011). Non-vaccinated virus-challenged Muscovy ducks also presented more severe clinical signs than Pekin ducks, suggesting differences in the innate immune response, and consequently the adaptive immune response, between these two duck species (Cagle et al., 2012).

The differences in the pathogenicity of H5N1 HPAI viruses in domestic ducks appear to be related to the species and not necessarily to the type or breed of the ducks. Muscovy ducks (*C. moschata*) presented more severe disease than various other breeds of domestic ducks (*A. platyrhynchos*), Pekin, Black

Runners, Mallards, Rouen, and Khaki Campbell, when infected with a H5N1 HPAI virus (Pantin-Jackwood et al., 2013). Mule ducks also appeared to be more resistant than Muscovy ducks to H5N1 HPAI virus infection (Ferreira et al., 2012). The variation in H5N1 HPAI pathogenicity and the differences in response to vaccination between different types of ducks should be taken into account when developing effective control measures for H5N1 HPAI in ducks, including surveillance, vaccines and vaccination strategies. With both killed and live vaccines, Muscovy ducks appear to be of special concern and likely require more booster vaccinations to achieve the same level of protection as seen in other ducks.

2.3.3. Effect of vaccination schedules

Different vaccination protocols have been examined in order to develop protocols compatible with husbandry practices. Ducklings in Asian countries are often reared indoors until 30 days of age and are then released in the open. Vaccines must therefore be administered during the confinement period (Beato et al., 2007). One-week-old Pekin ducks were vaccinated against H5N1 HPAI virus to determine if vaccination at this age would produce good protection if ducks were exposed 2 weeks later (Pfeiffer et al., 2010). Vaccination at this age, although conferring good protection against disease, did not stop virus shedding, and in certain cases, the ducks shed viruses for more than five days (Pfeiffer et al., 2010). In order to explore other alternatives for the efficient vaccination of ducks, studies were conducted using three different vaccination schedules to immunize Pekin and Muscovy ducks against H5N1 HPAI, two of which involved two doses of vaccine, given at 1 and 14 days of age or 7 and 21 days of age; and a third schedule using one dose given at 14 days of age (Cagle et al., 2012). The option that conferred the best protection was to vaccinate ducks at 7 days and 21 days of age. At 7 days of age, ducks in the field would also have decreased maternal antibody titers compared to one day old ducks, so would probably respond well to this vaccination schedule. Vaccinating ducks at one day of age followed by a boost at 14 days of age did not protect any better than vaccinating only at 14 days of age, the reason probably the immaturity of the immune system of the ducks at day of age (Cagle et al., 2012). However, another study using a two-dose vaccination program starting at day-old using an inactivated conventional vaccine, was successful in preventing clinical signs and mortality and in suppressing shedding of viable virus in vaccinated ducks (Beato et al., 2007), but it was not clear how much of this protection was conferred by the vaccination at one day of age. The authors also found a duration of immunity above threshold levels for more than 5 months in the vaccinated ducks, which appears to be less than what was generated with reverse genetics based vaccines (Tian et al., 2005), but this is longer than the economic life of a meat duck, which is approximately 4 months. A double dose of a bivalent vaccine, given at 1 day and at 3 weeks, protected ducks from disease and mortality, although only low antibody responses were induced and virus was re-isolated from some of the vaccinated ducks (Middleton et al., 2007). The results of these studies indicate that early vaccination could be used in the field to prevent primary introduction and secondary spread in naive ducks. Alternative protocols need to be explored to improve vaccination against H5N1 HPAI in domestic ducks under field conditions.

2.3.4. Role of homology between vaccine and challenge virus

Homology between viruses and vaccines is determined based on protein sequences of the HA. It should be kept in mind, however, that although there is a correlation between genetic and antigenic distance, it is also possible that specific genetic differences of only one amino acid lead to a substantial difference in antigenic distance (Smith et al., 2004). For influenza, it has been observed that

antigenic distance is linearly related to the logarithm of the HI measurement. This principle is the basis for the construction of antigenic maps in which antigenic distances are visualized. Human influenza vaccines are updated when there is an antigenic difference of at least 2 two-fold dilutions in the HI assay (Smith et al., 2004). It is not clear if such criteria can be applied to avian vaccines since there are many differences between avian and human vaccination practices including vaccination schedules and the use of different adjuvants (van der Goot et al., 2007).

Little is known about the effectiveness of genetically more distant vaccines in preventing infection, disease, and transmission of H5N1 HPAI in domestic ducks. Most of the vaccine studies used vaccines that had a high homology with the challenge virus (Table 1). In most experiments, both the HA of the vaccine and challenge strains belonged to the Eurasian H5N1 subtype, and these vaccines in some cases were able to completely prevent virus shedding (Middleton et al., 2007; Tian et al., 2005; Webster et al., 2006). Some studies used viruses and vaccines that were genetically more distant (Beato et al., 2007; Middleton et al., 2007). Middleton et al. used a vaccine with a H5 virus (A/Chicken/Italy/22A/98 H5N9) with a protein homology of the HA1 of ~89% with the H5N1 challenge virus, and found minimal virus shedding. Beato et al. used a vaccine based on the A/Duck/Potsdam/1402/86 H5N2 virus with a protein homology of the HA1 of 89% with the H5N1 challenge strain and did not isolate any virus. Not all currently used vaccines have such a high degree of homology with the circulating H5N1 field viruses. In one study, a widely used H5N2 vaccine strain that has a HA1 protein homology of 84% with the H5N1 HPAI challenge virus not only prevented severe morbidity and mortality but also significantly reduced virus shedding and transmission of H5N1 in ducks 2 weeks after vaccination (van der Goot et al., 2008). It is difficult to draw firm conclusions from these studies because of the differences in study design, vaccines, and challenge strains used. Current experience with vaccines used with chickens shows that the best protection results from vaccination with homologous vaccines, and the more divergent the vaccines are to the challenge strain the worse the protection based on morbidity, mortality, or viral shedding (Suarez, 2010). It is unlikely that vaccination in ducks would be different.

2.3.5. Novel vaccines, vaccine adjuvants, and vaccine markers

Conventional inactivated vaccines have proven useful in the control and prevention of H5N1 HPAI outbreaks in poultry. However, outbreaks still continue to occur in domestic ducks. New and improved vaccines and vaccination strategies are needed to adequately control H5N1 HPAI in ducks. Among the new approaches to vaccination is the use of novel virus-vectored vaccines. Poxvirus vectors, shown to be immunogenic in both Pekin and Muscovy ducks, have the advantage of being easily administered at the hatchery at 1 day of age (Bublot et al., 2010). In addition to the pox-vectored vaccines, new vaccines using duck enteritis virus (DEV) or Newcastle disease virus (NDV) as vectors have been developed and examined in ducks. Live attenuated DEV vaccine is used routinely to control lethal DEV infections in many duck-producing areas. Liu et al. (2011b) constructed two recombinant viruses in which the HA gene of a H5N1 virus was inserted within different sites of the DEV genome. Duck studies indicated that one of these constructs had a protective efficacy similar to that of the live DEV vaccine against lethal DEV challenge, and a single dose induced complete protection against a lethal H5N1 HPAI virus challenge in as little as 3 days post vaccination. These results suggest that recombinant DEV is suitable for use as a bivalent live attenuated vaccine, providing rapid and complete protection against both DEV and H5N1 HPAI virus infection in ducks, however the mechanism for the protection induced by the DEV-vectored vaccine still remains to be determined. A recombinant Newcastle disease virus (NDV) expressing

the HA gene of H5N1 influenza virus has been used as a bivalent vaccine against both NDV and H5N1 viruses in chickens in China since 2006 (Chen and Bu, 2009; Ge et al., 2007). In a study by Ferreira et al., a recombinant NDV vaccine was shown to elicit both humoral and cellular responses in 11-day old Mule ducks, correlating with a complete clinical protection against a H5N1 HPAI challenge. However, a suppressive effect on vaccine response from maternally derived antibodies (MDA) was observed (Ferreira et al., 2012).

An effective vaccine needs not only good antigens but also good adjuvants to enhance the immunogenicity of the antigen. Most vaccines for avian influenza are developed and optimized for chickens, but may not be optimal in ducks. However, one study in SPF ducks (species not identified) and chickens compared three different adjuvants using an avian influenza virus vaccine, and all three, mineral oil, Montanide™ ISA 70M VG, and Montanide™ ISA 206 VG, induced comparable antibody titers in both species (Liu et al., 2011a).

Another approach to enhance vaccine immunogenicity is to incorporate immune-stimulators like cytokines in vaccine constructs. In a study conducted by Qian et al., a recombinant fowlpox virus coexpressing the HA gene of a H5N1 HPAI virus and chicken interleukin 6 gene (rFPV-AIH5AIL6) was constructed and tested to evaluate the immune response in ducks (Qian et al., 2012). The rFPV-AIH5AIL6 vaccine induced a higher anti-AIV HI antibody response, an enhanced lymphocyte proliferation response, an elevated immune protection, and a reduction in virus shedding compared to a recombinant fowlpox virus expressing the HA gene alone. This study suggests that both cellular and humoral immunity contribute to better protection induced the rFPV-AIH5AIL6 vaccine, and that chicken interleukin 6 might be an effective adjuvant for increasing the immunogenicity of FPV-vectorized AIV vaccines in ducks (Qian et al., 2012). A similar study examined the immunogenicity and protective efficacy in Pekin ducks of a DNA vaccine encoding a chimeric protein of HA subtype H5 fused to CD154 (CD40L) (Yao et al., 2010). Immunization with this vaccine conferred protection against a genetically distant H5N1 HPAI virus but several doses of the vaccine were needed, which makes it impractical for use in the field. Taking a different approach to improving the immune response to vaccination, an enhanced immune response was observed in Vietnamese and Muscovy ducks fed Sophy β-glucan, a polysaccharide that potentiates the immune response, when vaccinated with a recombinant inactivated avian influenza H5 subtype vaccine (Le et al., 2011).

Considering the nature of the domestic duck industry in countries like China, Vietnam and Indonesia, and the difficulty with being able to identify vaccinated ducks, alternative DIVA strategies that could be used for serosurveillance in ducks when vaccination is used as part of a H5N1 HPAI control program are also being considered. One of these strategies involves the inclusion of an exogenous antigen in the vaccine that can be used as a positive marker for vaccination. The use of a tetanus toxoid (TT) marker has been evaluated in ducks given a H6N2 avian influenza vaccine (James et al., 2008). High levels of TT-specific antibodies, produced in twice-TT vaccinated ducks, persisted out to 19 weeks. There was no interference by inclusion of TT in an inactivated H6N2 vaccine for H6- or TT-seroconversion, indicating that TT could be a highly suitable exogenous marker for avian influenza vaccination in ducks (James et al., 2008). In another study, two H5 vaccines that could potentially be used in DIVA strategies were evaluated in ducks (Chua et al., 2010). The protective effect of a subunit avian influenza virus H5 vaccine based on recombinant baculovirus-expressed H5 HA antigen and an inactivated H5N2 avian influenza vaccine combined with a marker antigen (TT) were shown to confer protection in young ducks (Chua et al., 2010).

3. Conclusions

Vaccination strategies implemented in China and Vietnam were initially successful in controlling H5N1 HPAI; however, in time, outbreaks in poultry recurred. The practical difficulties of vaccination, as well as the rapid antigenic drift of H5N1 viruses, are likely to be responsible. The continued circulation of H5N1 HPAI viruses in many countries has resulted in the emergence of new strains, requiring that vaccine strains be updated each year or created for use in specific countries or regions. Results from field and laboratory evaluation of vaccines against H5N1 HPAI in ducks indicates that factors such as challenge virus, duck species and use, vaccination protocols, and proper use of vaccines may significantly influence the outcome of the H5N1 vaccination program. Other factors, including the role of maternally derived antibodies and co-infection with other pathogens, remain to be determined in ducks. Vaccine failure might occur due to several reasons, including low antigenic homology between the vaccine and the circulating strain, an insufficient viral antigen load in the vaccine, the use of an adjuvant not adapted for ducks, and/or not providing appropriate booster vaccinations. When assessing vaccine efficacy in field settings, strategies that account for differences between duck species may need to be implemented. Better matching of vaccines with circulating viruses, optimized vaccines and vaccination programs should improve the results of influenza immunization in ducks.

The role of vaccination needs to be considered in the overall H5N1 HPAI control strategy. Most vaccine programs are designed to prevent or reduce clinical disease with the ideal goal of eradicating a disease from a region. Currently, vaccines programs in endemic countries are being used for disease control purposes only because of lack of adequate coverage, lack of proper antigenically matched vaccines, and proper vaccination protocols. Although domestic ducks are key part of the epidemiology of maintenance and spread of H5N1 is Asia, the virus has not been a costly cause of disease for duck growers. Therefore farmers have been reluctant to invest in avian influenza vaccines when the primary value is for the greater veterinary and public health benefits. Greater attention to duck vaccination has to be considered if eradication of H5N1 HPAI is the ultimate goal. There is a need for effective vaccination strategies including vaccines suitable for mass vaccination. In countries where poultry is mainly backyard scavenger poultry, optimum vaccination coverage might be difficult to achieve. Vaccines may still prevent the host from showing clinical disease but fail to stop or adequately reduce virus shedding by the host. To effectively control the virus from circulating in poultry, an efficient post-vaccination surveillance program should also be established. This appears to be the main challenge for developing countries in the use of vaccination against AI. A control program should include strict quarantine, movement controls on animals and equipment, increased biosecurity, extensive surveillance, depopulation of infected animals, and a comprehensive education program for the public.

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